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Research Article

DETERMINATION OF PESTICIDE RESIDUES IN WATER AND SOIL SAMPLES FROM ANSIKHOLA WATERSHED, KAVRE, NEPAL

Babi Kumar Kafle[1], Balram Pokhrel[1], Sandeep Shrestha[2], Rosha Raut[2] and *Bed Mani Dahal[2]
[1]Department of Natural Sciences, Kathmandu University, Dhulikhel, POBox 6250, Nepal
[2]Department of Environmental Science and Engineering, Kathmandu University, Dhulikhel POBox 6250, Nepal
**Author for Correspondence*

ABSTRACT
Agricultural intensification helps to uplift socio-economic condition of farmers but the overuse of pesticides in farmland cause health and environmental problems. It is assumed that agricultural intensification contaminates river water and soil via pesticides applications. However, there are very limited studies on quality assessment of river water in reference to pesticide pollution in Nepal. The aim of the current study is to investigate the level of pesticide contamination in soil and water of agricultural intensive area (Ansikhola watershed, Kavre, Nepal). The analysis is focused mainly on organochlorine and organophosphorous as these two groups are most commonly used in Nepal. Endosulfan, iprobenfos, monochrotofos, mevinphos and butamifos are detected in water samples where as cypermethrin, dichlorvos, and cyafluthrin are detected in soil samples. The concentration up to 2 ppb has been reported in this study.

Keywords: *Agricultural Intensification, Pesticides, Organophosphate, Organochlorine, Synthetic Pyrithroate*

INTRODUCTION
The practice of farming in Nepal was traditionally organic in nature until the use of pesticides started in the early 1950s with DDT for malaria eradication (Thapa, 2002). The major pesticides used are organochlorines (such as BHC, dieldrin, chlordane), organophosphates (like ethyl parathion, methyl parathion, malathion, and oxydemeton methyl), carbamates and some synthetic pyrethroids (Neupane, 2001). As agriculture is more commercialized today, Nepalese farmers have started using various kinds of pesticides to increase yields. The use of pesticides helped to increase agricultural productivity but also created the adverse impact on human health and the environment (Shah *et al.*, 2009)
The high rate of population growth (CBS, 2003) and insufficient agricultural production to feed the growing population is one of the driving factors for agricultural intensification in Nepal. The national policy of Nepal has aimed to increase the per capita food production from 277 kg to 426 kg by 2017 is also contributing for intensive production (NPC, 1995). Agricultural intensification helps in uplifting socio-economic condition of farmers (Dahal *et al.*, 2009), however, the overuse of pesticide in the process causes environmental problems like land and water pollution and health problems of people (Ajayi, 2000; Atreya, 2005; Pimentel, 2005). The negative impacts of injudicious use of chemical pesticides on soil and water quality in global context are also recorded (Singh, 1994).
The average use of pesticides in Nepal is about 142 gm/ha (Shrestha and Neupane, 2002), which is small amount compared to other countries (Gupta, 2004). However, some of the agricultural intensive areas are recorded of applying 1.45 kg/ha (Sharma, 1994). The Indian subcontinent and China have experienced heavy use of organochlorine pesticides, such as hexachlorocyclohexanes (HCHs) and DDT, for agricultural purposes. It has been estimated that more than 10^8 kg of DDT has been used in India alone (Sharma, 1993) after its first initial formulation. Both India and Nepal have banned DDT for agricultural purposes, but it is still in use for malaria control (Sharma, 1993; Santillo *et al.*, 1997). It has been reported that benzene hexachloride (BHC) dust is the most frequently sold chemical pesticide followed by parathion methyl, zinc phosphide, aluminium phosphide, malathion, dithane, and phorate in Nepal (SKJ, 2003). Since most of these pesticides are extremely persistent chemicals, most likely they will persist in

the environment and impact adversely for years to come. The study area, which is one of the agricultural intensive area of the mid-hills region, has also relatively higher rate of pesticides use (Palikhe, 2001).

Most of the high doses of pesticides use is on cash crops such as sugarcane, paddy, potato, tomato and other vegetables (Kansakar *et al.,* 2002; Adhikari, 2004). Similarly, Pujara and Khanal (2002) reported the use of pesticides in significantly higher doses (higher than the recommended level) in cash crops like tomato and potato in Jhikhu Khola watershed. Furthermore, 92% of the households in Anshi Khola watershed use pesticides (Adhikari, 2004). It is obvious that the growing more commercial crops in Nepal mean more exposed to pesticides (Atreya, 2004).

The existing literature clearly shows the trend of increased use of chemical fertilizers and pesticides, especially in semi-rural and peri-urban areas, that have easy access to urban markets where a high demand for vegetables, fruits and other fresh produce exists year-round (Brown and Shrestha, 2000; Pujara and Khanal, 2002; Shrestha and Neupane, 2002). A long time before Manandhar (1997) has already reported on the tendency of increasing pesticide use in agricultural sector and in controlling insect borne diseases. For example, more than 1000 M Ton per year is consumed in agricultural sector where as about 560 M Tons of pesticides is used to control insects. The Pesticide Registration Office of Nepal boasts 63 companies and 3032 registered pesticide retailers. However, FAO and United Nations Environment Program (UNEP) study highlights on availability of more than 7000 hazardous chemicals with around 1500 new ones introduced every year (Poudel, 2004). Farmers are still using persistent organic pesticides such as DDT, endrin, and lindane (BHC) for different purposes in Nepal (Yadav and Lian, 2009). Organochlorine pesticides such as DDT and lindane (BHC) are still openly used in some pocket areas of the country such as Dhading, Bhaktapur and Kavre districts.

Soil and ground water are being polluted by the use of pesticides (Shah *et al.,* 2009). Shah *et al.,* (2009) further detected organochlorine and other pesticides including heavy metal in the soil sample of northern side of Amlekgunj in 2008. Similarly, Palikhe (2001) has raised the issue of water pollution due to pesticides use in Nepal. Because of the higher rate of pesticide use in agricultural intensive areas, it is expected that such pesticides especially organochlorine and organophosphates are present in the study area.

MATERIALS AND METHODS

Study Area

The study site, Ansikhola, is a small watershed (about 13 km^2) in the Kavre district of Nepal. It lies between N 27°41' to 27°44' latitude and E 85°31' to 85°37'30" longitude and the elevation varies from about 800 to 2000 meters above sea level (Figure 1). The sampling sites of Anshikhola are mentioned in Table 1.

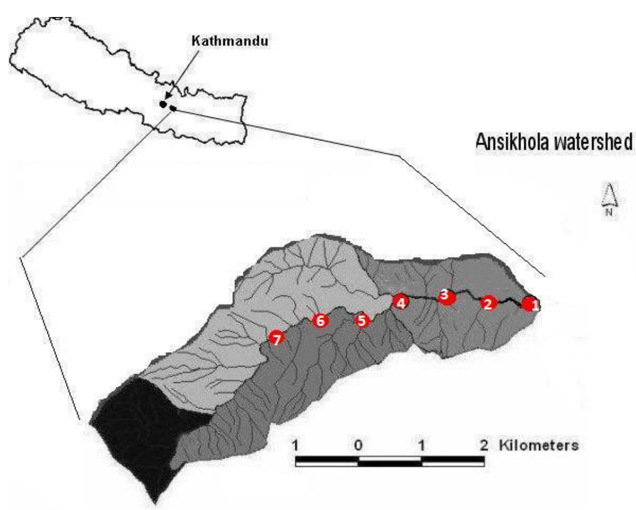

Figure 1: Water Sampling sites at Ansikhola watershed in Kavre district of Nepal

Water Sample Collection and Processing

The water samples from the stream of Ansikhola watershed were monitored in two seasons in order to check the types of pesticides use as per cropping pattern. It was expected that the two seasons monitoring would help to indentify and quantify whether the pollution is direct (application in same season and recorded on water sample) or indirect (record of pesticides which were not applied in that season). The first sampling (pre-monsoon) was carried out in March 2013 and second sampling (post-monsoon) was in November 2013.

From each sampling sites, one liter of water is collected from different points in cross-section of the stream (Red marks with sampling number in Figure 1). Since the velocity and geometry of the stream differ, the mixing of the materials in water differs and it is important to collect samples from all possible points of the stream section. The water sample is collected in the borosilicate glass bottle and preserved by adding 2ml of 5N H_2SO_4 acid. The samples were labeled and stored in icebox for transportation. Further treatments and processing of the samples were carried out in the laboratory of Kathmandu University.

The water sample process was followed as reported by Brondi *et al.*, (2011). In brief, 1L of sample water was transferred into a 2L separating funnel. Chloroform (10 ml) was added and shaken vigorously for 2 minutes. The supernatant was transferred quantitatively to a second centrifuge tube containing 4g $MgSO_4$, 1g NaCl, 1g disodium citrate dihydrate and 0.5g sodium citrate sesquihydrate. The tube was shaken vigorously again for 15 minutes. The supernatant was transferred and cleaned up using a commercial solid phase extraction cartridge containing 330 mg of primary secondary amines (PSA), 330gm of C18 and 1cm layer of $MgSO_4$ activated with 3ml of acetonitrile. The content was decanted and the chloroform layer was collected. The collected amount was concentrated to 2ml and transferred to sampler vials. Thus, prepared samples were analyzed by GCMS.

Table 1: The water and soil sampling sites in Ansikhola

Site No.	Location description	Geographical location (based on GPS recorder)
1	Ansikhola just before the confluence of Cha and Ansikhola, Siudinitar	27°42'20" N 85°36'55" E Elevation: 758±12 m
2	After the confluence of small stream, Dhaitar	27°42'21" N 85°36'26" E Elevation: 796±36 m
3	Beneath the suspension bridge and before the diversion canal, Dhaitar	27°42'22 N 85°36'03" E Elevation: 782±40 m
4	After the confluence of Sisnekhola, coming from left hand side, before the diversion canal, Piple	27°42'21" N 85°35'35" E Elevation: 802±21 m
5	10m before the gorge with hills in both sides & without agricultural lands for about 100m upstream & downstream.	27°42'14" N 85°35'26" E Elevation: 821±38 m
6	Bhotchaur, about 100m below the road crossing and after the confluence of other river coming from right hand side	27°42'15" N 85°35'12" E Elevation: 902±19 m
7	Mayal danda, bottom of the Anikot VDC (near two- three "Simal" trees)	27°42'08" N 85°34'48" E Elevation: 868±41 m

Soil Sampling and Processing

Application of pesticides has direct effect on soil and the application of pesticides may remain for longer than in water. Considering the fact soil samples were also considered for the study. The soils from the agricultural lands adjacent to the water sampling points were collected. Similar to water samples, the soil samples were also monitored in two seasons to check the types of pesticides use as per cropping pattern.

In the soil sampling process, first, five soil sampling points were identified in the land for representation of agricultural plot. From each point, about one kg of soil sample was collected from 0.1 square meter area. The soil was dug up to 0.15cm depth (plough height). The total 5kg of soil from five sampling

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points was homogeneously mixed and spread evenly in a paper. The soil was divided into four quarters and two opposite quarters were rejected. The remaining two quarters were mixed and spread into four quarters. Again, soils from two alternative quarters were rejected. This process was repeated so that about one kg of soil sample is left in the paper. The remaining one kg of soil sample was rapped with aluminum foil, locked in plastic zip bag, labeled and transported to the lab. The soil sample processing was followed as reported by Brondi *et al.*, (2011) and Nagel (2009). In brief, 10g soil was weighed into a 50ml centrifuge tube. 10ml acetonitrile was added and shake vigorously for a minute in vortex. The supernatant was transferred quantitatively to a second centrifuge tube containing 4g MgSO4, 1g NaCl, 1g disodium citrate dihydrate and 0.5g sodium citrate sesquihydrate. This tube was shaken vigorously again for 1 min. Thereafter the tube was centrifuged for 5 min at 3000 rpm and cooled down with ice water. The supernatant was transferred and cleaned up using commercial solid phase extraction cartridge containing 330 mg of primary secondary amines (PSA), 330 gm of C18 and 1cm layer of $MgSO_4$ activated with 3ml of acetonitrile. The extract was passed through the column and concentrated to 2 ml and transferred to the vial. The extract was analyzed by GCMS.

Chromatographic Conditions

The soil and water samples were analyzed by using GCMS QP 2010 Plus, Shimadzu. The spectra of standards and calibration curve from the instrument are presented in figure 2 and 3 whereas the major parameters set in the GCMS were as follows.

Oven Temperature Program:			
110°C at 2 min		Interface Temperature	280 °C
110°C - 150°C at 20°C/min for 1 min hold		Solvent Cut Time	2 min
150°C - 200°C at 4°C/min for 2 min hold		Start Time	3 min
200°C - 280°C at 5°C/min for 18 min hold		End Time	53.5 min
Ion Source Temperature	200 °C	Injection Mode	Split less
Injection Temperature	250 °C	Linear Velocity	28.3 cm/sec
Inlet Pressure	33.7 kPa	Purge Flow:	3.0mL/min
Total Flow	3.6 mL/min	Split Ratio	0.0
Column Flow	0.57 mL/min		

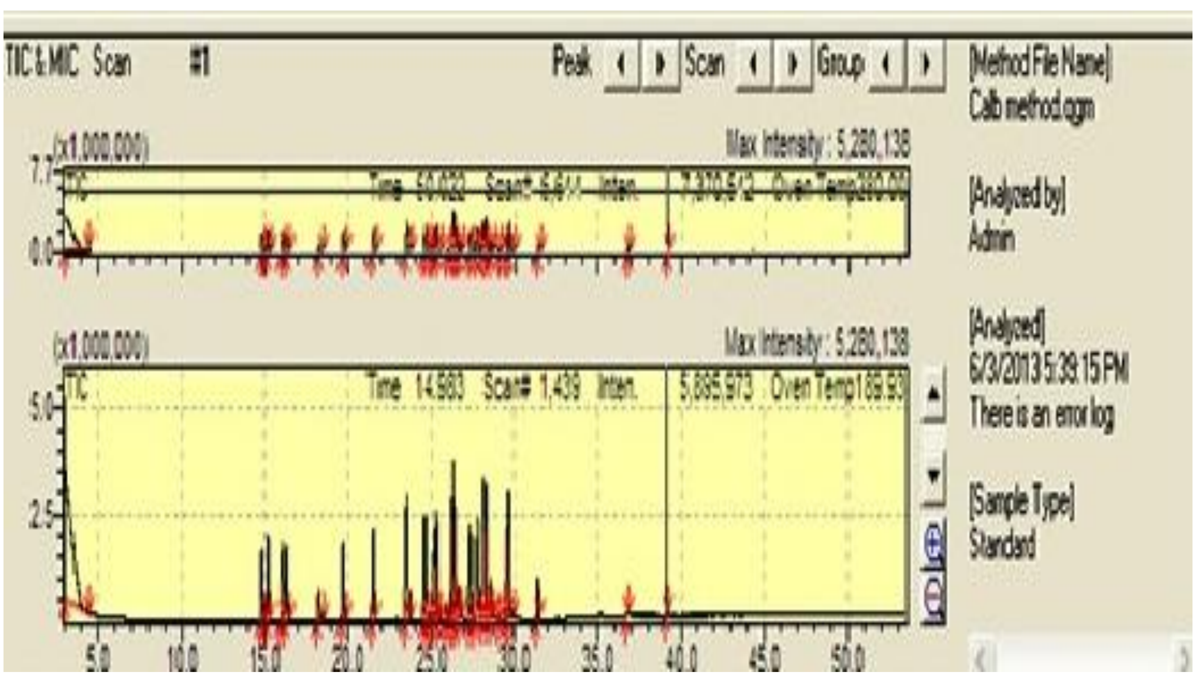

Figure 2: Spectra of standard from the GCMS

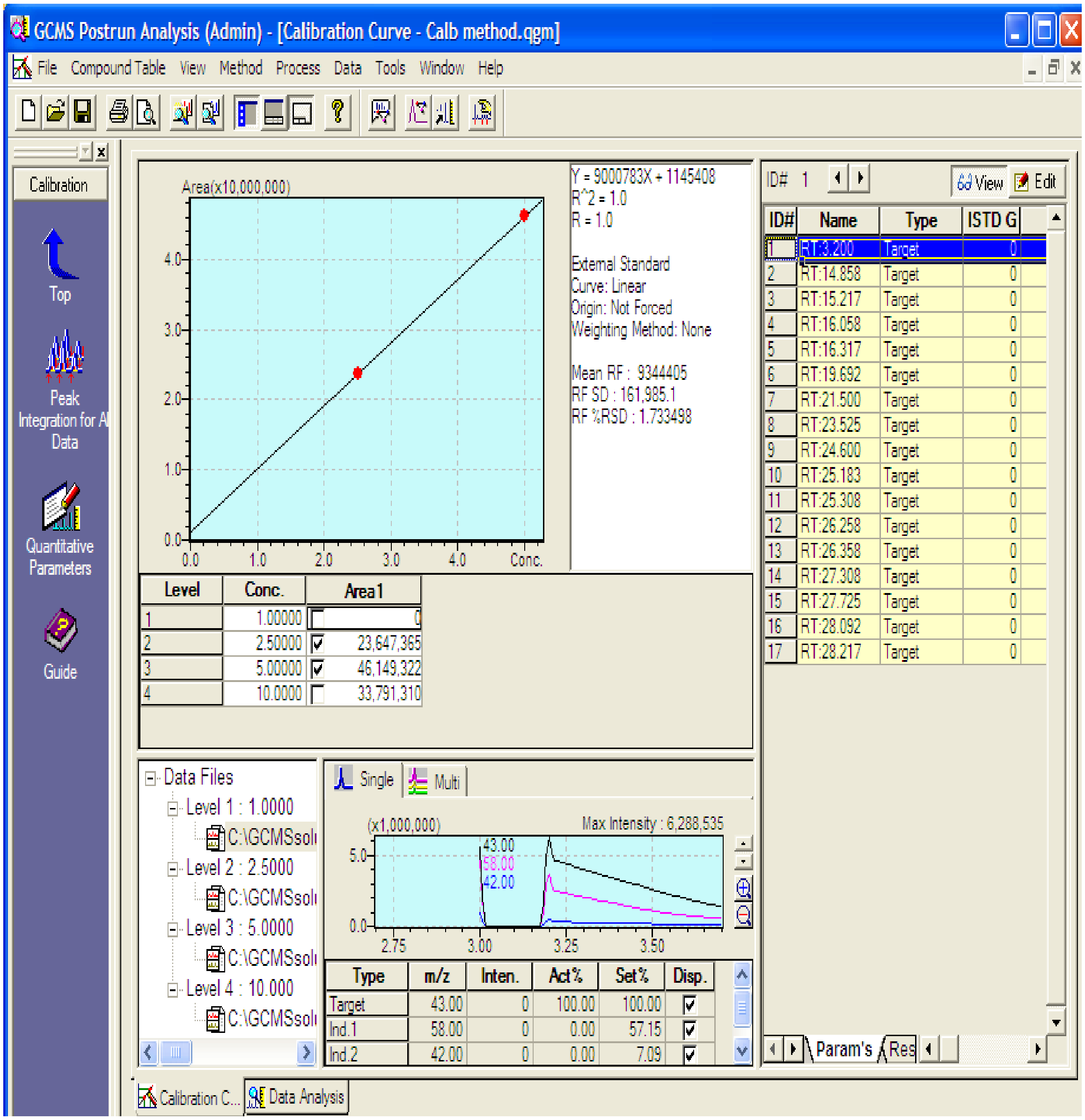

Figure 3: A calibration curve from the GCMS

RESULTS AND DISCUSSION

Pesticides Analysis in Soil and Water

The pesticides applied in the agricultural field are usually not detected directly in their original forms as they move from the place of application as well as change their form (Rand *et al.*, 1995). The study results also support their findings as most of the pesticides applied by the farmers (from questionnaire survey) are not detected in most of the soil and water samples. Some samples possess the residues of pesticide, which might be due to the transformation and persistent nature of the pesticides as highlighted on the properties of chemical pesticides by Belfroid *et al.*, (1998).

The analysis was mainly focused on organochlorine and organophosphate estimation. Hence, thestandards for the analysis used was Restek USA product for 19 pesticide mainly for organochlorine (DDT and its metabolite (o & p DDD /DDE)s, BHCs, Chlordanes, heptachlor, aldrin, dieldrin, endosulfans (alfa and beta), endosulfan sulfate, and organophosphate (19 pesticide mixtures), organ phosphorus (monochrotophos, triazophos, chloropyrifos) and the synthetic pyrithroates (cypermethrin).

Table 2: Results of the pesticides analysis in water samples

Sample Code	**Pesticide parameters**					
	Organochlorine: DDT and its metabolites (o and p DDD /DDE), Aldrin, Dieldrin, Endrin, Heptachlor, BHCs, Chlordane, Endosulfans		**Synthetic Pyrithroate: Cypermethrin**		**Organophosphporus: Chlorfenviphos, Chlorpyriphos, Triazophos**	
	I*	**II****	**I***	**II****	**I***	**II****
Wa-1	BDL	BDL	BDL	BDL	BDL	BDL
Wa-2	BDL	BDL	BDL	BDL	BDL	Iprobenfos: 191.0 µg/l
Wa-3	BDL	BDL	BDL	BDL	BDL	BDL
Wa-4	BDL	Endosulfan Sulfate: 50.0 µg/l	BDL	BDL	BDL	BDL
Wa-5	BDL	BDL	BDL	BDL	BDL	Iprobenfos: 3980 µg/l
Wa-6	BDL	BDL	BDL	BDL	BDL	Iprobenfos:318 µg/l; Monochrotofos:118 µg/l; Mevinphos:103 µg/l; Acephate:43 µg/l
Wa-7	BDL	BDL	BDL	BDL	BDL	Butamifos: 3980 µg/l

*Note: * Pre-monsoon sampling (March); **Post-monsoon sampling (November); BDL = Below Detection Limit (< 2.0 µg/l).*

The results obtained from the analysis of water and soil samples have shown the presence of organochlorine, synthetic pyrithroate and organophosphate pesticides. The compounds detected were endosulfan sulphate as organochlorine; cypermethrin and cyafluthrin as synthetic pyrithroate; iprobenfos, monochrotofos, mevinphos, acephate, butamifos, and dichlorvos as organophosphate pesticides. The detail result of the analysis is presented in Table 1 and Table 2.

Among the various pesticides analyzed, only cypermethrin, cyafluthrin and dichlorvos were found in the soil samples whereas endosulfan sulphate, iprobenfos, monochrotofos, mevinphos, acephate and butamifos were detected in water samples. The highest concentration (3980 µg/l) of iprobenfos and butamifos was detected in water samples of site 5 and 7 respectively, which was sampled during post monsoon period. This might be due to the application of those pesticides in paddy fields as during the sampling time, the paddy fields were ready to harvest.

Table 3: Results of the pesticides analysis in soil samples

Sample Code	**Pesticide parameters** **Organochlorine: DDT & its metabolites (o and p DDD /DDE), Aldrin, Dieldrin, Endrin, Heptachlor, BHCs, Chlordane, Endosulfans**		**Synthetic Cypermethrin**	**Pyrithroate:**	**Organophosphporus: Chlorfenviphos, Chlorpyriphos, Triazophos**	
	I*	**II****	**I***	**II****	**I***	**II****
So-1	BDL	BDL	BDL	BDL	BDL	BDL
So-2	BDL	BDL	Cypermethrin: 125.1 μg/kg	BDL	BDL	BDL
So-3	BDL	BDL	BDL	Cypermethrin: 3342 μg/kg	BDL	BDL
So-4	BDL	BDL	BDL	BDL	BDL	Dichlorvos: 390 μg/kg
So-5	BDL	BDL	BDL	BDL	BDL	BDL
So-6	BDL	BDL	BDL	Cyafluthrin: 1888 μg/kg	BDL	BDL
So-7	BDL	BDL	BDL	BDL	BDL	BDL

*Note: * Pre-monsoon sampling (March); **Post-monsoon sampling (November); BDL = Below Detection Limit (< 2.0 μg/kg).*

According to Hamilton *et al.*, (2004), the levels of malathion, lindane and dieldrin in fresh water should not be more than 0.1, 0.08 and 0.36 μg/l respectively. However due to the detection limit of the instrument, such chemicals could not be detected below 2 μg/l in the study. It cannot be inferred that the sites where pesticides were not detected are free from pesticides as those might had lower concentration than the detection limit. The samples collected during post monsoon contained the higher concentration of organophosphate pesticides in comparison to pre-monsoon. This can be attributed to the monsoon run off from the catchment area of intensive agricultural activity. The higher concentration of detected pesticides like iprobenfos and butamifos in river water might be due to their higher solubility in water, lower affinity to be adsorbed in soil and their wash down effect of runoff. Similarly, the detection of endosulfan sulfate and dichlorvos at sampling site 4 in water and soil respectively might be due to the application of "thiodin" (endosulfan) and "nuvan" (dichlorvos) in paddy fields. This result is supported by the application of higher dose of "thiodin" by farmers in paddy as reported in the survey. In spite of the lower the concentration of these pesticides the observed residues were higher than the Environmental Protection Agency 0.01 μg/L (USEPA, 2000) for protection of aquatic life.

Conclusions

The level of organophosphate pesticides was found to be higher than organo chlorinated pesticides in both soil and water samples of Ansikhola watershed. The concentrations of the organophosphate pesticides alone are lower in soil samples than in water sample. The existence of these pesticides at levels known to be higher than the tolerable limits adopted by (EPA) for survival of aquatic organisms clearly indicates the ecological risk associated with the exposure of those species and the likelihood of their being transferred along the food chain and the danger their continued increase usage portends to human health. Pesticide degradation is limited due to rapid transportation through cracks and macropores in loamy soils and most of the study area have loamy soils. Hence, applied pesticides are detected higher in stream water. More detailed monitoring studies need to be conducted to study the leaching behavior of these pesticides in stream as well as ground water.

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REFERENCES

Adhikari B (2004). Agricultural pests, their control and impacts of pesticides on environment in mid-hill watershed of Kavre district. M. Sc. Thesis, Department of Biological Sciences and Environmental Science, Kathmandu University, Nepal.

Ajayi OC (2000). Pesticide use practices, productivity and farmers' health: the case of cotton-rice systems in Cote d'Ivoire, West Africa. Pesticide Policy Project special issue publication series no 3, University of Hanover, Germany.

Atreya K (2004). Farmers using pesticides are unaware of the dangers and alternatives, Nepal, GDNet document store, 2004.

Atreya K (2005). Health costs of pesticide use in a vegetable growing area, central mid-hills, Nepal. *Himalayan Journal of Sciences* **3**(5) 81-84.

Brondi SHG, Macedo AN de, Vicente GHL and Nogueira ARA (2011). Evaluation of the QuEChERS Method and Gas Chromatography–Mass Spectrometry for the Analysis Pesticide Residues in Water and Sediment. *Bulletin of Environmental Contamination and Toxicology* **86**(1) 18-22.

Brown S and Shrestha B (2000). Market-driven land-use dynamics in the middle mountains of Nepal. *Journal of Environmental Management* **59** 217-25.

CBS (2003). National Sample Census of Agriculture, Nepal, 2001/02: highlights. National Planning Commission, HMG Nepal, Kathmandu. pp. 45.

Dahal BM, Nyborg I, Sitaula BK, and Bajracharya RM (2009). Agricultural intensification: food insecurity to income security in a mid-hill watershed of Nepal. *International Journal of Agricultural Sustainability* **7**(4) 249-260.

Gupta PK (2004). Pesticide exposure-Indian scene. *Toxicology* **198** 83-90.

Hamilton D and Crossley S (2004). *Pesticide Residues in Food and Drinking Water: Human Exposure and Risks* (John Wiley & Sons, Ltd).

Kansaka VS, Khanal NR and Ghimire M (2002). Use of insecticides in Nepal. In: *Proceedings of International Workshop on Environmental Risk Assessment of Pesticides and Integrated Pest Management in Developing Countries, Kathmandu, Nepal 6-9 November 2001*, edited by Hermann A and Schumann S 90-98.

Manandhar D (1997). *Sportlight* **23** (35) Mar. 19-25.

Nagel TG (2009). The QuEChERS Method - a new approach in pesticide analysis of soils. *Journal of Horticulture, Forestry and Biotechnology* **13** 391.

Neupane FP (2001). Synopsis of Use and Economic Value of Pesticides and Chances for Reduced Application Rates at Different Scales in Hindu Kush Region, CEAPRED Report, Kathmandu.

NPC (1995). *The Eighth Plan (1992-1997)* (National Planning Commission, Kathmandu).

Palikhe BR (2001). Pesticide pollution management in Nepal in harmony with nature. In: *Agriculture and Environment* (HMG/Ministry of Agriculture and Cooperatives) Kathmandu, Nepal.

Pesticides Registration and Management Section (2006). Pesticides registration and management program: introduction, achievements and directives (in Nepali). Ministry of Agriculture and Cooperatives, Department of Agriculture, Nepal. pp.79.

Pimentel D (2005). Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability* **7** 229-52.

Poudel K (2004). Toxin-hit Nepal to curb pesticide use. One World South Asia, March 10, 2004.

Pujara DS and Khanal NR (2002). Use of pesticides in Jaishidhi subcatchment, Jhikhu Khola Watershed, Middle mountain in Nepal. In: *Proceedings of International Workshop on Environmental Risk Assessment of Pesticides and Integrated Pest Management in Developing Countries, Kathmandu, Nepal 6-9 November 2001*, edited by Hermann A and Schumann S 168-177.

Santillo D, Johnston P and Stringer R (1997). *Pesticide News* **36** 4.

Shah BP and Devkota B (2009). Obsolete pesticides: their environmental and human health hazards. *The Journal of Agriculture and Environment* **10** 51-56.

Sharma D (1993). *Pesticide News* **21**15.

Sharma KC (1994). Current Experiences and Practices in Pesticide Use in the Bagmati Zone, ADPI Series No.9, ICIMOD, Kathmandu, Nepal.

Shrestha PL and Neupane FP (2002). Socio-economic context on pesticide use in Nepal. *Landschaftsökologie und Umweltforschung* **38** 205-223.

Singh P (1994). Land degradation - A global menace and its improvement through agro-forestry. In: *Agroforestry Systems for Sustainable Land Use*, edited by Panjab S, Pathak PS and Roy MM (Oxford and IBH Publishing Co.) India 283.

SKJ (2003). Pesticides poisoning in Nepal. *Kathmandu University Medical Journal* **1**(3) 157-157.

Thapa RB (2002). Pesticide pollution and integrated pest management. In: *Integrated Pest Management in Nepal*, edited by Neupane FN (Himalayan Resources Institute) Kathmandu, Nepal 175-194.

USEPA (2000). *Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates*, 2nd edition (EPA boo/R-9910064, Office of Research and Development Washington, DC).

Yadav SK and Lian KK (2009). The management of persistent organic pollutant pesticides in Nepal. *Macquarie Journal of International and Comparative Law* **6** 217-236.